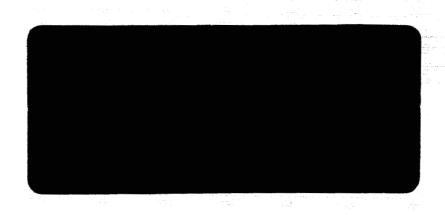
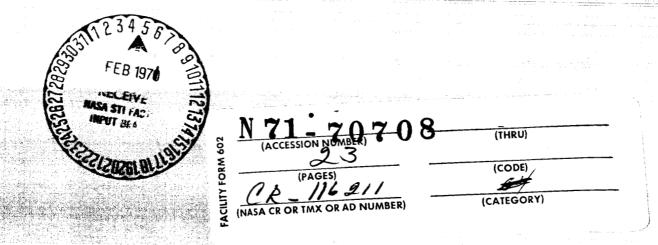
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JET PROPULSION LABORATORY

CALIFORNIA INSTITUTE OF TECHNOLOGY

PASADENA, CALIFORNIA

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VOYAGER MARS PLANETARY QUARANTINE:

MICROMETEOROID SIMULATION EXPERIMENTAL STUDIES - STATUS REPORT

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STATUS EXPORT

Introduction

This document reports the status of the experimental program being conducted to evaluate the effects of micrometeoroid impaction on spececraft surfaces and the subsequent potential for contamination of the planet Mars. Micrometeoroids impacting unsterile spacecraft surface materials will cause the release of unsterile ejecta; creating a potential source of contamination.

The experimental program essentially consists of subjecting typical spacecraft materials, intentionally seeded with a known level of biological contemination, to hypervelocity impacts. The simulated micrometeoroids are cast iron projectiles with an average diameter of 5 microns, traveling at 30,000 feet per second. The ejecta from the target materials is collected in a gelatin medium for subsequent biological assay.

The General Electric Biosciences Operation - MOL Department, is responsible for the target preparations and post-test biological assay operations, as well as the maintenance of sterility of specimens and apparatus throughout the test program.

The hypervelocity impact test facility is located at Morgantown, Pennsylvania, and is directed and operated by the General Electric Space Sciences Laboratory.

This experimental study is now essentially complete. The early test activities, conducted to establish the feasibility of this program, have been reported in the General Electric Company, Voyager, Task C Bi-Monthly

Reports Nos. 1, 2, and 3. The formal experimental effort was defined as a 32-test series in Bi-Monthly Report No.4. Early status of this test series was presented in Document No. VOY-C2-TM 10. As of the end of January, 1967, the 32 tests have been completed. Preliminary bio-assay data from these tests is reported herein. Data reduction and analysis is still continuing and the final results will be released in a later publication. The interpretation and evaluation of this data, from the standpoint of potential Hars contamination, will become a part of the overall Voyager Task C Planetary Quarantine Sensibivity Studies.

STATUS REPORT

EFFECTS OF SIMULATED MICROMETEOROID IMPACT ON MICROBIOLOGICALLY CONTAMINATED SURFACES

A. Summary

The objective of these simulated micrometeoroid impact tests is to determine the number of viable microorganisms released from an artificially contaminated target. Four materials, gapresenting those spacecraft surfaces which will be predominantly exposed to micrometeoroid impact, have been tested; 6061 aluminum, 2024 aluminum, Textolite, and fused silica. Testing to date has been completed on all target materials. These materials were tested, prepared with a low level (10² - 10⁴) and with a high level (10⁷ - 10⁸) of spores of <u>Bacillus subtilis</u> var. <u>niger</u> per target.

Preliminary evaluation of the results indicate that from <1% to about 40% of the innoculated spores may be released in a viable state from the impacted side due to impact. However, 75% of the data indicate this range to be between 1% and 10%. Further analysis of these data should provide information as to the effect of the target material and thickness as well as whether there is a correlation between the numbers of viable organisms released from the materials and the number of particles impacting the material.

In several of the earlier documents referred to in the Introduction, it was explained that hypervelocity impacts may produce three types of ejecta sources; splash, spall and what we have termed "table banging". Splash and spall are both directly related to the crater produced at impact and are,

Textolite - Fiberglass epoxy; Tradename of the General Electric Company, Chemical and Metallurgical Division, Conshocton, Ohio.

for all practical purposes, impossible to view separately. On the other hand, the ejecta released from areas remote to the impact via internal vibrations set up by such impact, table banging, offers some room for separate evaluation. Tasts have been conducted, in which the size of the contaminated area was varied, to evaluate this effect.

Lastly, several tests have been conducted to determine the size distribution of the non-biological particulate ejected due to the hypervelocity impacts.

Non-contaminated targets were tested, using the same test apparatus as described herein. The gelatin collection medium was replaced by paraffin for these tests to simplify recovery and examination of the particulate ejects.

B. Experimental Apparatus

Diagrams of the testing apparatus (Microparticle Projector) and collecting chamber (Micrometeoroid Trap) are presented in Figures 1 and 2, respectively. Figure 3 is the aluminum block used to form the cone in the upper (top) of the collecting chamber. Figures 4 and 5 are photos of the apparatus. The description of and background for the test materials selected have been previously presented in Document No. VOY-C2-TM-10, dated December 1966.

Microbiological testing, sterilization of targets and apparatus, inoculation (artificial contamination) of targets and post test assays were all carried out at General Electric's Bioscience Laboratory, Valley Forge, Pennsylvania.

C. Preparation Prior to Firing

1. Target materials are "bio-cleaned" and sterilized in dry heat at 160°C for a minimum of 8 hours and then stored in sterilized petri dishes prior to inoculation.

An aqueous suspension of spores of <u>Bacillus subtilus</u> var. <u>niger</u> are sonicated for 10 minutes in an ultrasonic bath (Appendix A) containing cold water and cracked ice. An aliquot of the suspension (0.01 milliliters) is assayed to determine the level or number of spores actually deposited on the target. Contamination is accomplished by depositing approximately 0.01 milliliters of spore suspension onto the center of the target. The targets are seeded equally on both sides. This procedure is followed for each set of four tests run at the same contamination level and on the same day. The droplet is allowed to air-dry and forms a contaminated area which is approximately 0.02 square inches. Aqueous stock spore suspensions are held in a refrigerator at 4°C to prevent or retard germination. After inoculation the targets are stored in presterilized petri dishes until ready for insertion into the testing apparatus.

2. The Microparticle Projector (Figures 1 and 4) is also "bio-cleaned" and sterilized with ethylene oxide (12% ethylene oxide, 88% freom (dichlorodifluoromethane) prior to each test.

According to the techniques for cleaning stainless steel strips in NASA Standard Microbiological Examination of Spacecraft Hardware, June 1966, National Aeronautics and Space Administration, Washington D.C.

The testing apparatus (Microparticle Projector) is wrapped in a polyethylene bag and sealed by folding over the top portion of the bag three folds and stapling it closed. It is placed in a sterilizing chamber and humidified for one hour to assure the penetration of the water vapor into the plastic bag. At the end of the humidification, a vacuum is drawn on the chamber to evacuate the air. Ethylene oxide is slowly introduced into the chamber until a pressure of five pounds is reached (~ 600 mg. RTO/1 of chamber space). The apparatus is exposed for a minimum of 17 hours. When sterilization is completed the chamber is evacuated and the system is allowed to equilibrate with the atmosphere. Air is introduced into the chamber through a .45y membrane filter. The apparatus is carried to the testing site in the sterilized polyethylene container. Sterility is maintained until the beg is opened for the aseptic mounting of the Micrometeoroid Trap (Figures 2 and 5). The internal sterility is maintained until the instant of the firing of the explosive charge. The reason for the breach in sterility is that the charges are not sterilized; there is no effective means of maintaining sterility during the required handling in preparation of the charge. (Swabbing and fallout plates have not shown evidence of the typical orange pigmented colonies which might be confused with the test organisms (Bacillus subtilus var. niger).

3. The Micrometeoroid Trap is "bio-cleaned" and sterilized in the same manner as the testing apparatus (sterilized by ETO treatment in polyethylene bag scaled with Time Tape 1). The micrometeoroid trap is

¹ Manufactured by Professional Tape Company, Inc., 355 Burlington Ave. Riverside, Illinois.

designed to collect both front (top) and back (bottom) face ejecta from the targets. Twelve percent gelatin² is used as the collecting medium. The 12% collecting gelatin is sterilized at 121°C (250°F) for 15 minutes and 15 pounds pressure. A sterile aluminum cone (Figure 3) is placed in the top collecting chamber and 30 milliliters of sterile gelatin is poured around it. The entire unit is placed in a refrigerator at 4°C to harden the gelatin. The bottom half of the trap is filled with 20 milliliters of gelatin and placed in the same refrigerator. When the gelatin is hardened the aluminum cone is removed and the contaminated target is mounted between the collecting chamber halves (Figure 2).

D. Firing of Charge

Prior to firing of the charge, a vacuum is drawn on the microperticle projector to between 5 2 and 10 m absolute pressure. After the firing, the collecting trap is placed in a sterile polyethylene bag and the bag scaled with taps. The trap is then returned to the microbiological laboratory for assay.

E. Post-Firing Assay Procedure

The gelatin is aseptically removed from the collecting trap; the top cone portion (30 milliliters) is placed in 70 milliliters of 1% peptone water to make a total volume of 100 milliliters. The bottom collecting gelatin (20 milliliters) is placed in 80 milliliters of 1% peptone water to make a total volume of 100 milliliters. The gelatin and water solutions are

Produced by Difco Laboratories, Detroit, Mich., or Fisher Scientific Company, Manufacturing Chemists, Fairlawn, H.J.

³ Bacto-Peptone "Difco Certified" Difco Laboratories, Detroit 1, Michigan

five minutes. At the completion of the ultrasonic treatment, the samples are diluted for quantitative assessment of the microorganisms recovered. The samples are assayed according to the standard plate count technique (ref. APHA)¹. Trypticase Soy Agar² is the plate count medium. Plates are incubated in an inverted position at 37°C and observed for microbial colony development at 24, 48 and 72 hours.

F. Results

The formal test series of 32 tests has been completed. A compilation of the direct assay results to date is presented in Tables 1 and 2, representing the numbers of organisms recovered as a result of release from the front face (top) and back face (bottom) of the targets, respectively. In the tests at a low contamination level, the number of viable test spores recovered was extremely small and the data at these levels is consequently less significant. It appears that at the 10⁴ level we have obtained more significant recoveries (See data for fused silica, Tables 1 and 2).

Impact areas are being measured and analysis will be made to see if they correlate with the number of organisms released from the targets. The targets are also being assayed to determine the number of viable organisms remaining on their surfaces (top and bottom). Reduction of this data in conjunction with the initial level of contamination, will provide some measurement as to the numbers lost or killed due to particle impacts.

American Public Health Assoc., Standard Methods for the Examination of Water and Waste Water, pp. 492-3, Amer. Public Health Assoc., Inc., 1790 Broadway, New York 19, N.Y.

² Trade Hame of Baltimore Biological Laboratories, Baltimore, Maryland.

G. Supplementary Tests and Test Results

TABLE BANGING LIALUATION

Four tests were conducted to determine the degree of table banging present in the experimental test setup. One-eighth inch thick aluminum targets were employed. Each of the four tests were identical except that the area of contamination was increased for each test. Contamination areas ranged from 0.07 to 0.7 square inches. The spore suspension, used for contamination in all four tests, had a spore density of 1.3 x 10⁸ spores/0.01 milliliters. Consequently, as the contaminated target area increased, so did the total number of spores present on the target increase. Table 3 shows the data from these tests.

Figure 6 depicting the number of organisms recovered vs. the contaminated area, indicates an apparent increase in recoveries with increasing contamination area. Any extrapolation in the lower end of the curve is totally unsupportable. Two factors contribute to the increasing recovery with increasing area; more inoculated organisms and a larger area permitting more hits. Note that the point at about 0.5 in² contaminated area is off the curve. In conjunction, note the far fewer hits (71) on this area. Apparently, the number recovered is more closely related to the number of hits, than to the area contamined, as seen in Figure 7.

Figure 8 incorporates the determination of hit areas for each target. The hit area is the sum of the areas of all craters. Dividing the number of recovered organisms by the hit area for any one target, puts the recovery assay data on a common basis (1); accounting for the uncontrollable variables of number of hits and hit sizes.

⁽¹⁾ Assumes a uniform distribution of spores over contamined area.

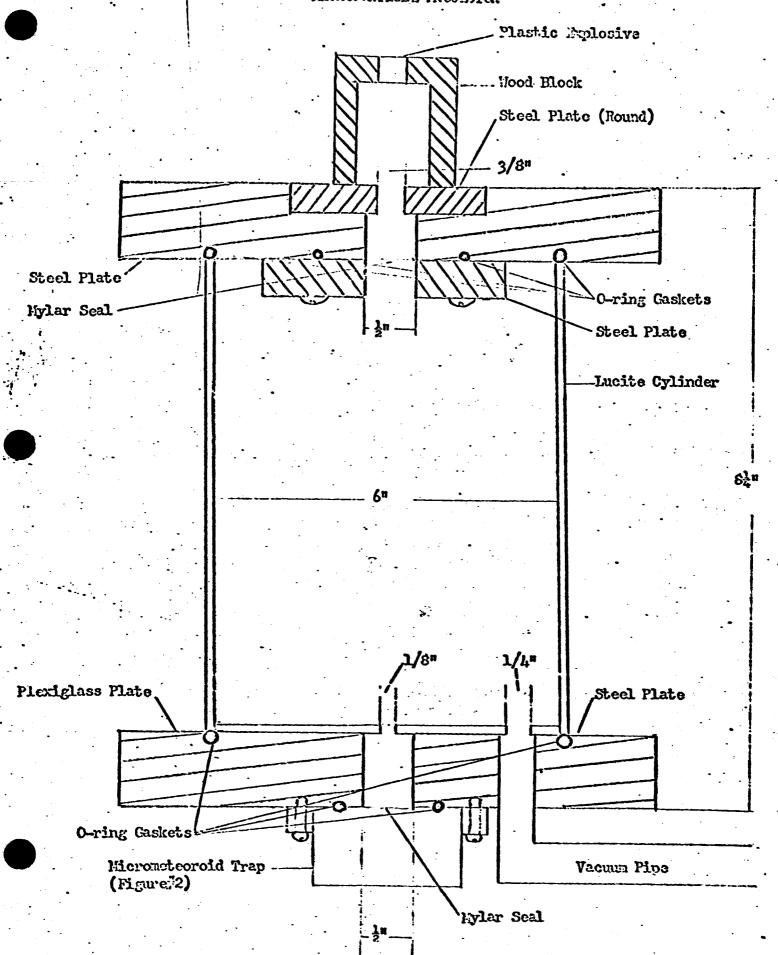
Note that in Figure 8, recovery per hit area is relatively constant regardless of the size of the contaminated area. Further evaluation of these results is underway.

DETERMINATION OF EJECTA SIZE DISTRIBUTION

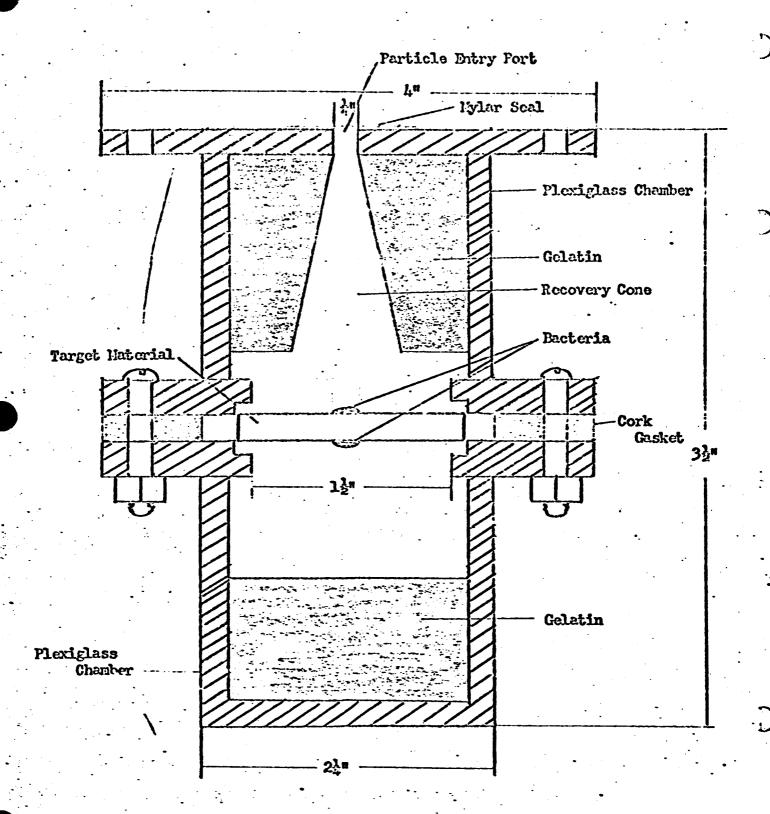
The experimental apparatus described herein and shown in Figures 1, 2, 4 and 5 was used to evaluate the ejecta size distribution. In this series of eight tests no biological contamination was employed. The gelatin collection medium was replaced by paraffin since there were no biological constraints imposed and the paraffin was more amenable to examination.

The same four target materials were tested, namely 6061 and 2024 aluminum, Textolite and fused silica. Ejecta from both the impacted (front) face and back face were collected and microscopically counted. The test data is shown in Table 4. It may be noted that about 75% of the ejecta, consisting of target material, cast iron from the projectiles, and detonating charge debris, is less than five microns in major dimension.

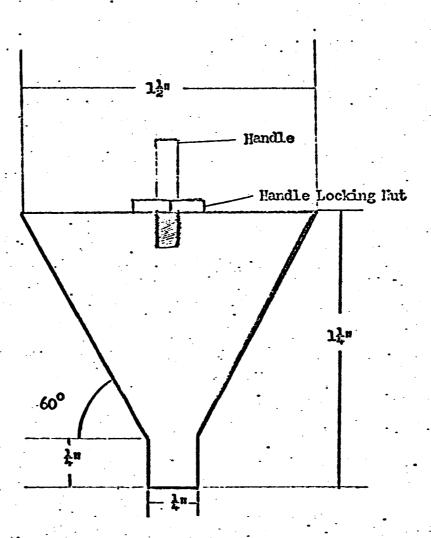
HICROPARTICLE PROJECTOR

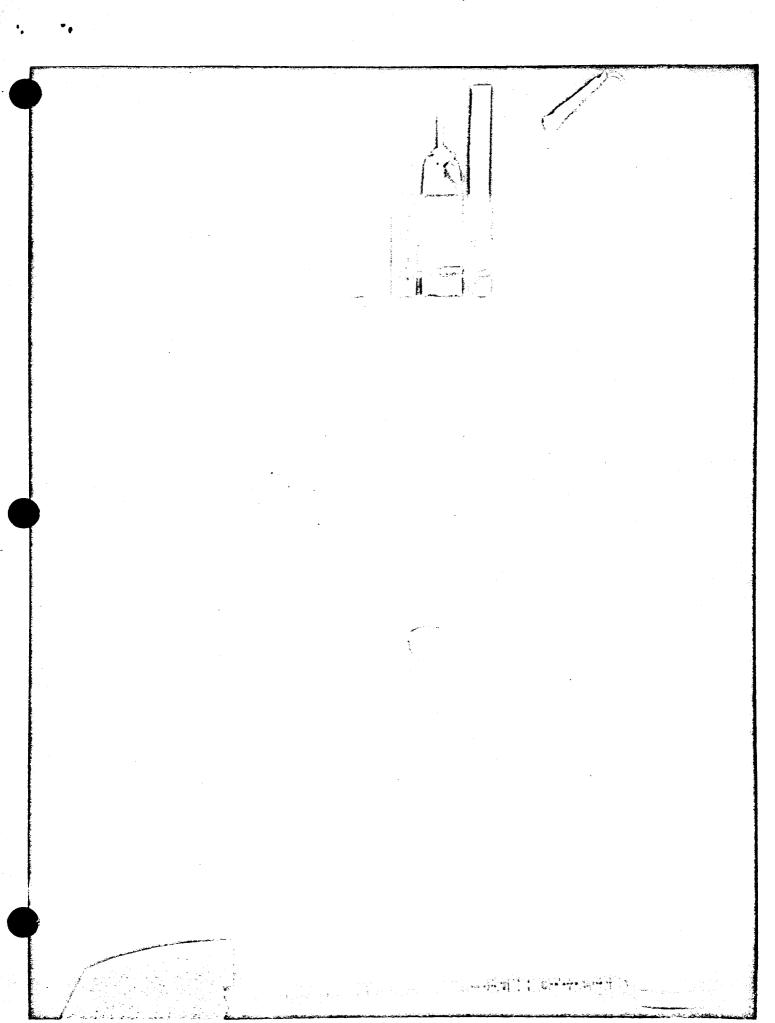


HICRCITTACION TRAP

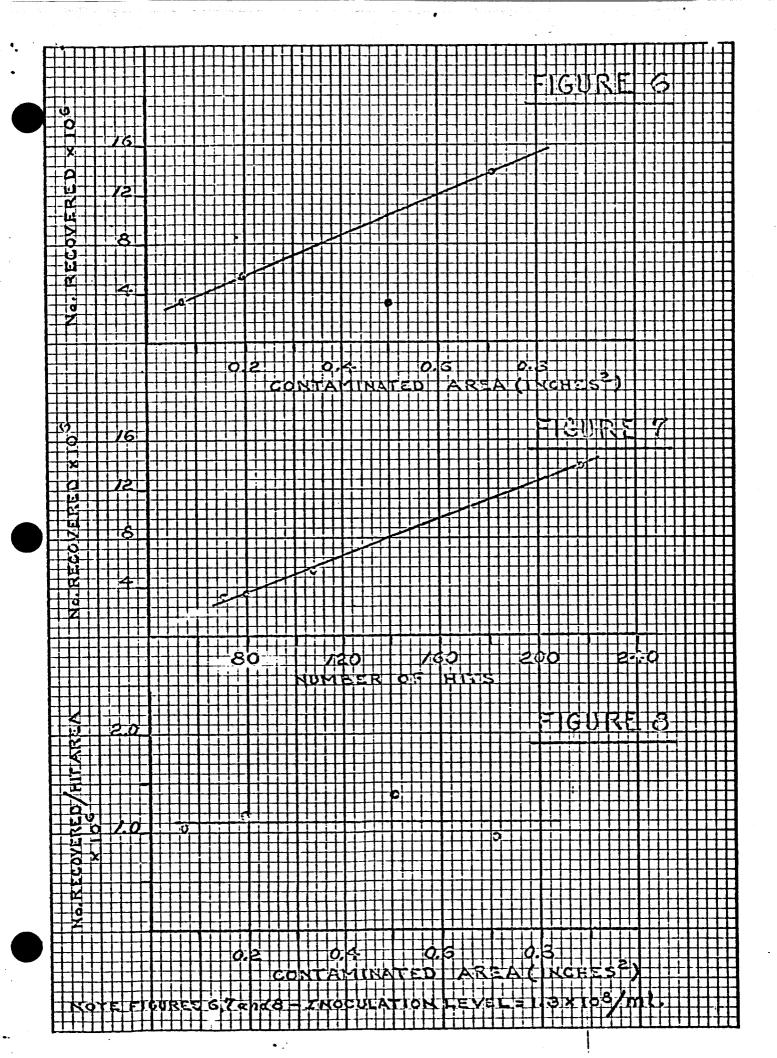


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Summary of Lovels of Organisms Recovered From Trupped Ejecta Prodused By Similated Micrometeoroid Impact on Actual Spacecraft Materi TOP SURFICE CRIX

322.36 6.60010 4.43220 , 53×10 3.5720 0532 6.272.0 33510 Pused Silica (元 元) 3,17×104 3.17200 3.17200 3,17510 9.6200 9.620 9.6000 0.650 1.94220 5,12005 5.775:05 2,625107 4.77500 2.73×10 2,0200 9,02304 4.000 4,Cx10 Textolita (12 min) 5.892103 5.89203 5.89×10 8 क्या 5.89210 S. 6x107 5.05×10 8,6210 5.05×10 5.05×10 5.05220 MATERIALS 5.51210 2,285107 9.73×10° 7.23×10 3.92005 1,902.10 5,0000 1.52107 1.52104 2,0000 2021, 11 uninum 日間の 1,062208 1,065008 2,93×102 1.06×108 2,93×102 1.53×107 1.53×10° 2.93×10⁴ 1,06×10 2.93320 1.95:202 1,63:10⁴ 7.15005 3.67:206 1.22205 3.73×10 1.56:10 1.52107 1.5:20 0 6061 Aluminum (TH 95) 1.65210 8 1.80×105 1.80000 1.652108 1.65×108 1.500106 1.652008 6,22102 6.22102 6.22102 6.220% tine permits) (conducted as Intermodiste Inoculation Low Lord High Lovel Level (10,-01) (10⁷-10⁸) Levele

* KEY- 1) Inco. Level/ Post Test Assay 2) All Targets contaminated on both sides

3.0010

\$ 1.7×10⁴

1,53300

2.3.204

Lost

L.90000

1.53300

1,1220

1.80210

Top side of aluminum targets coated with themmal rowers nature 3) Assay not related to hit area 4) Top side of aluminum targets

Summary of Levels of Organisms Recovered From Trapped Ejecta Produced By Similated Eicromoteoroid Impact on Actual Spacecraft Materials

borron surrace our

		Mused Silica (15 mil)	4.23×107 9.6×10 1.92×107	.07×107 9.6×10/	6,2720 5 9,620 7,5720 7		\ ₁ 0	101	3.0210 3.17210 5.03210	4,5210 1 3,17210 4 1,83x10	0035.1	2,5×10	5,1:103	nost	Assay not related to hit area 12th thermal Top, side, of All tergets coated with thermal
•	3	Textolite (15 mil)		\$. \$		\	\						8,6213	8,6200	3) Assay n 4) Ton sid
	14ATTATA1.S**	2024 Aluminum (3 mil)	1.062210 3.332407	1.06010	8 , 60, 11	1.06×10 5.03×10 7	2.93×10 2.5×10	2.93×10 0	2.93×10 1.5×10	2,93×10	90126-6	1.553×10 1.46×10	3.2205	1.53×20.4 2.44×10.4	Post Test Assay conteminated on both sides
	:	6061_Aluminum (11m 0,1)	1,65200 5,62007	1.65×108	1.652108	11	6,2210	6.200	6.2210	5.0	1,14405	1.30x10 2.20x10 4	!! \	1.82104	* KEY_ 1) Inoc. Level/Post
		Inoculation Levels		Hen Level	(91 • 91)	A CONTROL		Low Lovel	(97- (11)			Intermediate Level	(conducted as time permits)		

TABLE 3
VARIABLE AREA TESTS

Number of Hits	HI† Area (In ²)	Number Recovered (x10 ⁶)	Recovered per HIT Area (x10 ⁹)	Contam. Area (In ²)	Number Innoculated (x10 ⁸)
60	0.0032	3.3	1.03	0.0755	2.6
108	0.0047	5.5	1.19	0.1960	5.2
71	0.0023	3.2	1.39	0.5030	7.8
217	0.0149	13.7	0.92	0.7090	10.4
·	1	•	3	•	

TABLE 4
PARTICLE SIZE DISTRIBUTION

		\$ Per Size Range in Microns				
Material	Ejecta Source*	0-5	5-10	10-15	>15	
6061 AL, 40 mll	Тор	76.5	15.0	6.0	2.5	
	Тор	78.0			3.0	
	Bottom			NE -		
•*	Bottom	NONE				
2024 AL, 3 ml1	Тор	71.5	18.0	5.5 .	5.0	
. *	Тор	85.5		1.5	1.0	
	Bottom	73.5	16.0	4.5	6.0	
	Bottom	70.0	18.0	7.0	5.0	
Fused Silica, 15 mli	Тор	84.5	12.0	2.0	1.5	
	Тор	81.0		3.0		
	Bottom	75.0			6.5	
	Bottom	81.0	14.5	2.0	2.5	
Textolite, 15 mil	Тор	82.0	13.5	1.6	3.5	
	Тор	89.0	8.0	2.0	1.0	
	Bottom	77.5		3.0	3.0	
•	Bottom	73.0	14.0	4.0	9.0	
		•		f	•	
		•	•			

^{*}Top refers to the impacted, or <u>front</u> face of the target. Bottom refers to the <u>tack</u> face.

APPEIDIX A

ULTRASONIC BATH

The ultrasonic bath (Branson Instruments: generator, A-300 tank, LT-80; gas power control, PC-30; or equivalent) shall conform to the following specification:

- (1) The frequency shall be 25 kc/sec.
- (2) The power output in relation to bottom surface area of the tank shall be at least 2.3 w/sq. inch (0.35 w/cm²).
- (3) If the ultrasonic bath is not automatically tuned, tuning shall be performed according to the manifecturer's directions.
- (4) The inside surfaces of the bath shall be steinless-stell.
- (5) Glass bottles containing liquids or piece parts shall be supported on the bottom of the tank.
- (6) The temperature of the bath fluid shall be at least 25°C and shall not exceed 37°C. The bath fluid shall be changed periodically in order to prevent heat-up. To maintain a cool bath use cracked ice.
- (7) The both liquid shall be at least 1 inch above the level of the liquid in the bottles being ultrascalcated.